Rate of Cross-Fertilization Between Single Plants and Between Plots

Wolfgang Link and Ernst von Kittlitz
University of Hohenheim, Postfach 70 05 62,
7000 Stuttgart 70,
FEDERAL REPUBLIC OF GERMANY

Abstract

In field trials, the cross-fertilization between single plants and between small plots was studied in *Vicia faba* L. var *minor* using black-seeded marker-gene. The rate of cross-fertilization between single plants (42%) was about twice the rate between plots (22%). The results show that cross-fertilization is too frequent to be ignored.

Introduction

Faba bean (*Vicia faba* L.) is a partially allogamous species, a fact that renders its breeding difficult. Multiplication of genotypes in the open field causes problems of impurity in the genotypes for subsequent test trials, unless adequate isolation is provided. The problems that arise from this practice are:

1. A given genotype cannot be properly maintained.

2. Genotypes show different degrees of cross-fertilization. Therefore, even stocks with an equal coefficient of inbreeding will produce progenies with differing coefficient of inbreeding. This creates differences between the productivity of these progenies which are found at the homozygous level, thus decreasing the validity of the results.

3. The contamination with pollen from the whole nursery reduces the genetic variation between the stocks, and subsequently the heritability and the gain from selection.

To minimize these disadvantages, breeders often discard seeds from the border rows. Hawtin and Omar (1980) found, that harvesting the center of a plot (size of 15 m^2 or 30 m^2) instead of the borders, reduced contamination from outside the plots by 3 to 5%. Omar and Hawtin (1981) found a difference of about 6% between the samples from the border and from the central part of a plot; the difference was somewhat more pronounced when the experiment was located in the center of the field than when at the edge. Dietrich *et al.* (1979) reported from repeated experiments that their four-row-plots produced about 28% of the progeny pollinated from outside the plot, whereas single-row-plots produced 38%. However, Xanthopoulos *et al.* (1986) reported a rate of cross-fertilization within rows of 48% compared to only 8% between rows using single row plots with a row spacing of 0.7 m. Robertson and Cardona (1986) suggested, that neighboring plots of *Brassica napus* L. potentially reduced bee activity in the faba bean plots; however the rate of inter-plot outcrossing was not significantly reduced.

Pope and Bond (1975) found that an isolation distance of 0.9 m resulted in 17% contamination with 20 m^2 plots. However, contamination was reduced to 3% at 23 m, to 2% at 46 m, and to less than 1% at 138 m distance to the source of foreign pollen. Similarly, Hawtin and Omar (1980) reduced contamination from 7% (at plot spacings of 10-40 m) to less than 2% (at plot spacing of 200 m) with 6.25-m^2 plots. Also, they found that contamination was reduced as the plot-size increased.

The objective of the present work was to determine the rate of cross-fertilization between plants (i.e., within plots) and between small plots at different levels of heterozygosity of the faba bean genotypes at Hohenheim, Stuttgart, Germany.

Materials and Methods

Four experiments were conducted in the open field at Hohenheim, Stuttgart, Germany, during the 1985-1987 seasons to study the rate of cross-fertilization in faba bean (*Vicia faba* L. var *minor*). Nine genotypes of faba bean expressing the recessive buff seed color were used: three homozygous lines (line 03 from the variety Diana, line 20 from Herz Freya, and line 43 from Kleine Thueringer), along with the three possible F_1s and the corresponding F_2s.

The rate of cross-fertilization was measured with a top cross by examining the offspring in the topcross-test. The topcross-tester was a bulk of 16 lines, homozygous for the dominant black seed-color. The flowering period of the topcross-tester covered the flowering period of the nine genotypes.

In the topcross-test-generation the progeny of the nine buff-seeded genotypes was sown and number of black seeded and buff seeded progenies was counted. The rate of cross-fertilization (RCF) in the four experiments was calculated as follows:

$$RCF = \frac{\text{No. of black-seeded progenies}}{\text{Total no. of scored progenies}} \times 100(1+R)$$

where R is the relation of buff-seeded plants to black seeded plants (topcross-tester) during the flowering period in the topcross-generation.

Experiments 1 - 3

Experiments 1 and 2 included all the nine genotypes, whereas experiment 3 included only the three lines. The topcross-generations of the experiments were grown in 1985 (exp. 1), 1986 (exp. 2), and 1987 (exp. 3) at different fields. The genotypes were randomized in two blocks. Each plot consisted of 10 buff-seeded plants, surrounded by eight plants of the topcross-tester. The resulting plant density was 20 plants/m^2 with all plants equally spaced. An average of 1495 offsprings/genotype and experiment were scored in the respective topcross-test.

Experiment 4

The topcross-generation of experiment 4 was grown in 1987 at the same field as exp. 3. The buff-seeded genotypes were planted as whole plots (2x0.9 m^2 size) with three rows and 60 plants/plot. The relation of "black" plots to "buff" plots was 3 : 1 (**Fig. 1**). The "buff" plot were harvested in three parts: the two border rows (treatment 1), the five border plants of each side of the central row (treatment 2), and the center of the central row (treatment 3). An average of 486 offsprings/genotype and treatment were scored in the topcross-test.

"Total rate" of cross-fertilization was estimated in experiments 1 - 3, whereas in exp. 4, the rate of cross-fertilization between plots was estimated ("rate between plots"), i.e., the part of progeny which is fertilized by pollen from plants of neighboring plots. The "rate between plots" is the "total rate" minus the cross-fertilization between plants within the same plot.

Results and Discussion

A summary of the rate of cross-fertilization in experiments 1 - 4 is shown in **Table 1**. There was a strong negative influence of the degree of heterozygosity on the rate of cross-fertilization, and the mean of the F_2s was nearer to the mean of the parental lines than to the mean of the F_1s.

There were clear differences between experiments 1 - 3 and exp. 4. The "rate between plots" was, on average, about half of the "total rate," indicating that approximately half of the cross-fertilization occurred between plants within the same plot. These results are at variance with those of Xanthopoulos *et al.* (1986).

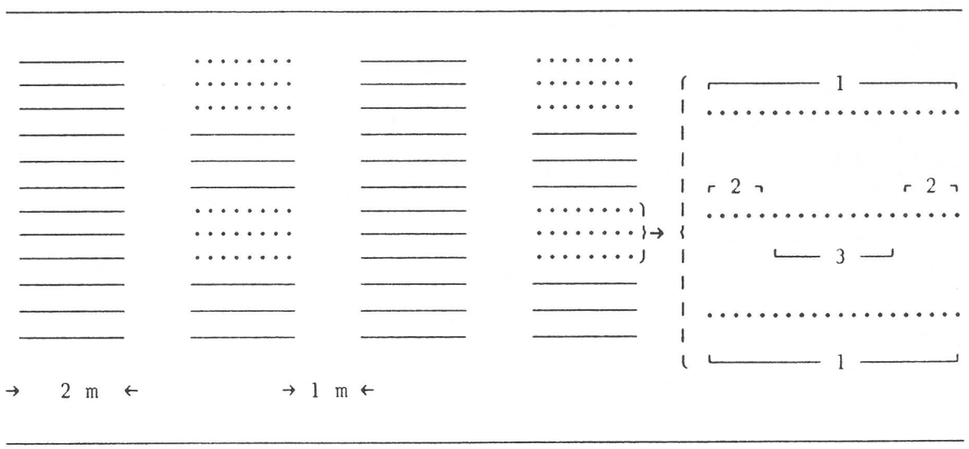

Fig. 1. Field design of experiment 4 (the solid lines symbolize a row of black-seeded plants and a dotted line symbolizes a row of buff-seeded plants; 1, 2, 3 correspond to treatments 1, 2, and 3).

Table 1. Rate of cross-fertilization faba bean genotypes (experiments 1 - 4) grown at Hohenheim, Stuttgart, Germany, during the 1985-1987 seasons.

Experiment	Generation /genotype									Mean
	P			F_2			F_1			
	03	20	43	03x20	03x43	20x43	03x20	03x43	20x43	
Exp. 1	57.3	42.6	51.7	49.2	53.2	41.9	37.1	26.3	11.7	41.2
Exp. 2	57.8	50.5	64.7	46.0	52.3	46.1	25.5	30.8	11.7	42.8
Exp. 3	64.1	58.1	40.4							
Mean[a]	59.7	50.4	52.3	47.6	52.8	44.0	31.3	28.6	11.7	
Mean		54.1			48.1			23.8		
Exp. 4										
Treat. 1	36.5[d]	38.4	35.0	30.9	22.9	30.0	18.4	14.1	4.8	25.7[c]
Treat. 2	38.5	30.9	30.3	25.0	29.9	22.3	10.2	12.1	6.2	22.8
Treat. 3	29.9	23.4	28.1	17.9	24.7	14.6	10.8	10.5	10.2	18.9
Mean[b]	35.0	30.9	31.1	24.6	25.8	22.3	13.2	12.2	7.1	22.5
Mean	32.3			24.2			10.8			

a. LSD (0.05) for comparison of genotypes: 11.5
b. LSD (0.05) for comparison of genotypes: 6.2
c. LSD (0.05) for comparison of treatments: 2.8
d. LSD (0.05) for comparison of genotypes within treatments: 6.6

In exp. 4, only small differences were detected between buff-seeded plants harvested from the center of the plot and those harvested from the border of the plot. Nevertheless, the influence of the treatments was significant. These results agree with the work of Omar and Hawtin (1981).

The effect of cross-fertilization on the coefficient of inbreeding of the stocks is shown in **Table 2**. Consider the self-pollinated progeny of a single, heterozygous plant, giving an F_2-plot as in exp. 4. The F_2-plants produced 48% of its progeny by cross-fertilization. However, only 24% was traced back to pollinators from other plots and subsequently were not inbred (coefficient of inbreeding of 0.00), whereas the remaining 24% was pollinated by F_2-plants within the same plot (coefficient of inbreeding of 0.50). Consequently, 52% of the progeny resulted from self-fertilization (coefficient of inbreeding of 0.75). The average inbreeding coefficient of the entire progeny is then 0.51 **(Table 2)**. Harvesting only the plants from the center of the plot did not markedly

Table 2. Average coefficient of inbreeding of the progeny of lines, F_2's and F_1's harvested from the whole plot or from the central part only.

Harvested area	Generation		
	P	F_2	F_1
Whole plot	0.68	0.51	0.38 (0.45)[a]
Central part	0.73	0.54	0.38 (0.45)

a. If all F_1-plants in the plot have the same genotype.

improve the coefficient of inbreeding, especially when compared with a coefficient of inbreeding of 0.75, achievable in bee-proof conditions.

Generally, it can be concluded that the rates of cross-fertilization between plots were too large to be neglected. A single row at each side of a plot is a weak shelter against foreign pollen and does not

diminish the rate of cross-fertilization within the plot. Thus, to reduce contamination with foreign pollen, additional sheltering rows should be planted from seed of the respective genotypes harvested from the open field at the previous year. The "inner" plot with true-bred seed will then yield a less contaminated progeny for larger trials.

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معدل الإخصاب الخلطي بين نباتات منفردة، وفيما بين قطع تجريبية

ملخص

ضمن تجارب حقلية على الفول، تمت دراسة الإخصاب الخلطي بين نباتات منفردةوبين قطع تجريبية صغيرة، وذلك باستخدام مورِّث علام ذي بذرة سوداء. وكان معدل الإخصاب الخلطي فيما بين النباتات المنفردة (42٪) حوالي ضعف ما هو عليه بين القطع التجريبية (22٪) . وهذه النتائج تظهر أن الإخصاب الخلطي كان شديد التكرار بحيث لا يمكن تجاهله.